William S. Klug
Trenton State College at Hillwood Lakes
Michael R. Cummings
University of Illinois, Chicago

CONCEPTS OF GENETICS

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Part Opening Photos

Part One (pp. 10–11): Light micrograph of root tip cells in various stages of division. (Courtesy of John McLeish.)

Part Two (166–67): Scanning electron micrograph of a portion of a lampbrush chromosome. (Courtesy of Nicole Angelier.)

Part Three (pp. 260–61): Electron microscopic visualization of a bacteriophage.

Part Four (pp. 378–79): Electron microscopic visualization of the process of transcription of DNA (RNA synthesis). (Courtesy of Oscar Miller.)

Part Five (pp. 530–31): Golden poppy. (Grant Heilman Photography.)

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day, there is no doubt of the validity of this conclusion; nor was there any reason to suspect otherwise.

The strongest evidence has been provided by a current experimental procedure called **recombinant DNA research.** In this procedure, segments of eukaryotic DNA corresponding to specific genes are isolated and literally spliced into bacterial DNA. Such a complex can be inserted into a bacterial cell and its genetic expression monitored. If a eukaryotic gene is selected that is absent in bacterial genetic information, the presence of the corresponding eukaryotic protein product demonstrates directly that this DNA is functional in expressing genetic information. This has been shown to be the case in numerous instances. For example, the human genes specifying the hormone insulin and the immunologically important molecule interferon are produced by bacteria following recombinant DNA procedures.

As the bacterium divides, the eukaryotic DNA is replicated along with the host DNA and is distributed to daughter cells. As divisions continue, the eukaryotic genes are **cloned,** with each new bacterial cell containing an identical copy of the eukaryotic genetic information. As a result, large amounts of DNA containing specific eukaryotic genes may be isolated and studied in depth. This technique has paved the way for detailed analysis of the nucleotide sequence of specific genes, among other types of information.

The availability of vast amounts of DNA coding for specific genes has led to a second form of documentation that DNA serves as the genetic material. In the work of John Gurdon and others, billions of copies of specific genes were injected into oocytes of the frog *Xenopus laevis.* In these experiments, the egg served as a living test tube, and the transcription and translation of this genetic information were analyzed. Through such analysis, the products of these genes have been identified, confirming the informational role of DNA in genetic processes.

More recent work in the laboratory of Beatrice Mintz and others has strengthened this evidence. This research has demonstrated that DNA encoding the human β-globin gene, when microinjected into a fertilized mouse egg, is later found in the adult mouse tissue and can be transmitted to that mouse's prog-

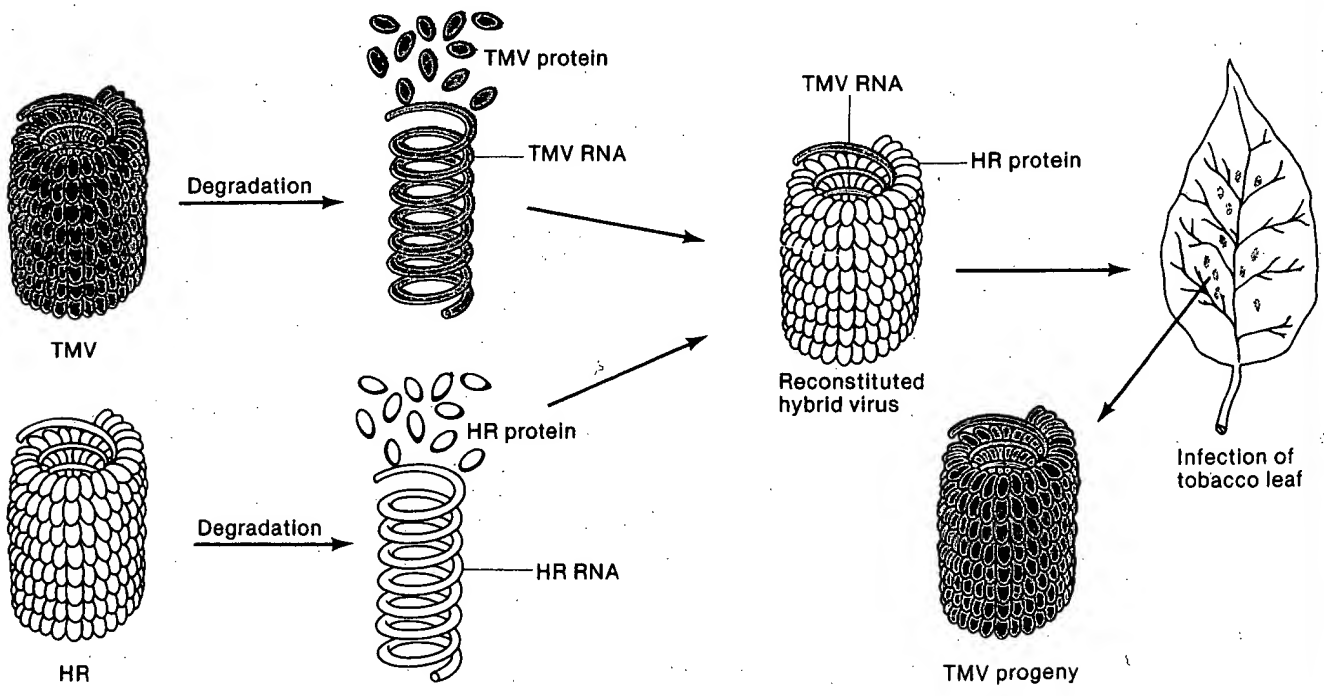

FIGURE 8.8
Reconstitution of hybrid tobacco mosaic viruses. In the hybrid, RNA is derived from the wild-type TMV virus, while the protein subunits are derived from the HR strain. Following infection, viruses are produced with protein subunits characteristic of the wild-type TMV strain and not those of the HR strain.

Such mice are referred to as **transgenic.** More recent work has introduced DNA representing the growth hormone gene from rats into fertilized mouse eggs. About one-third of the resultant animals grew to twice the size of normal mice, indicating that the foreign DNA was present *and* functional. Subsequent generations receive the gene and display the trait that it governs.

We will pursue the topic of recombinant DNA again later in the text. The point to be made here is that in eukaryotes DNA has been shown directly to meet the requirement of expression of genetic information. Later we will see how DNA is stored, replicated, expressed, and mutated.

RNA AS THE GENETIC MATERIAL

Many plant viruses and some bacterial and animal viruses contain an RNA core rather than one composed of DNA. In these viruses, it would thus appear that RNA must serve as the genetic material—an exception to the general rule that DNA performs this function. In 1956, it was demonstrated than when purified RNA from **tobacco mosaic virus (TMV)** was spread on tobacco leaves, the characteristic lesions caused by TMV would appear later on the leaves. It was concluded that RNA is the genetic material of this virus.

Soon afterwards, another type of experiment with TMV was reported by Heinz Fraenkel-Conrat and B. Singer. These scientists discovered that the RNA core and the protein coat from wild-type TMV and other viral strains could be isolated separately. In their work, RNA and coat proteins were separated and isolated from TMV and a second viral strain, **Holmes ribgrass (HR).** Then, mixed viruses were reconstituted from the RNA of one strain and the protein of the other. When this "hybrid" virus was spread on tobacco leaves, the lesions that developed corresponded to the type of RNA in the reconstituted virus; that is, viruses with wild-type TMV RNA and HR protein coats produced TMV lesions (Figure 8.8) and vice versa. Again, it was concluded that RNA serves as the genetic material in these viruses.

In 1965 and 1966, Norman R. Pace and Sol Spiegelman further demonstrated that RNA from the phage Qβ could be isolated and replicated *in vitro.* Replication was dependent on an enzyme, **Qβ RNA replicase,** which was isolated from host *E. coli* cells following normal infection. When the RNA replicated *in vitro* was added to *E. coli* protoplasts, infection and viral multiplication occurred. Thus, RNA synthesized in a test tube can amply serve as the genetic material in these phages.

SUMMARY AND CONCLUSIONS

The existence of a genetic material is deducible from the observed patterns of inheritance in organisms. The functions of the genetic material are replication, storage, expression, and mutation of genetic information. Geneticists considered both proteins and nucleic acids as candidates for the genetic material. In the first part of the twentieth century, proteins were favored because protein chemistry had developed faster than nucleic acid chemistry. Proteins appeared to be more functionally diverse than nucleic acids, and Levene's tetranucleotide hypothesis resulted in an underestimation of the role of the nucleic acids.

By 1944, the tetranucleotide hypothesis was invalidated, and evidence establishing the importance of DNA in genetic processes began to accumulate. Except for those viruses containing only RNA, DNA was shown to be the genetic material through experimental and circumstantial evidence. The direct experimental evidence was derived from certain critical investigations designed to answer the specific question: Of what substance is the genetic material? These experiments included work on transformation of bacteria and infection of bacteria with bacteriophages or DNA from bacteriophages. Beyond reasonable doubt, these experiments established the genetic role of DNA in bacteria and most viruses.

Initially, only circumstantial evidence was available for eukaryotes. It included (1) the distribution of DNA in tissues; (2) the correspondence of the UV-absorption spectrum of DNA and the action spectrum for UV-induced mutagenesis; (3) the metabolic stability of DNA compared with other major classes of biomolecules; and (4) the localization of DNA in cellular organelles for which a genetic function has been established (nuclei, chloroplasts, mitochondria). Recent recombinant DNA techniques have now provided direct experimental evidence that DNA serves as the genetic material in all eukaryotes.

An important exception to the general rule involves a variety of viruses where RNA serves as the genetic material. These include certain bacteriophages as well as some animal and plant viruses.

The establishment of DNA as the genetic material paved the way for the expansion of knowledge in molecular genetics. In this regard, the research that went into this finding has served as the cornerstone for further important studies over the past three decades.

GENETIC EXCHANGE AND RECOMBINATION IN BACTERIA

The development of the isolation techniques just described and the information derived from the subsequent study of bacterial mutagenesis led to detailed investigations of the arrangement of genes on the bacterial chromosome. These studies were initiated in 1946 by Joshua Lederberg and Edward Tatum. They showed that bacteria undergo **conjugation**, a parasexual process in which the genetic information of one bacterium is transferred to and recombined with that of another bacterium. Like meiotic crossing over in eukaryotes, genetic recombination in bacteria led to methodology for chromosome mapping.

Two other phenomena, **transformation** and **transduction**, also result in the transfer of genetic information from one bacterium to another. These processes also have served as a basis for determining the arrangement of genes on the bacterial chromosome. Transformation (see Chapter 8) involves the entry and integration of a piece of DNA from one bacterium into the chromosome of another intact organism. Transduction, on the other hand, is a phage-mediated transfer of small pieces of DNA from one bacterial cell to another.

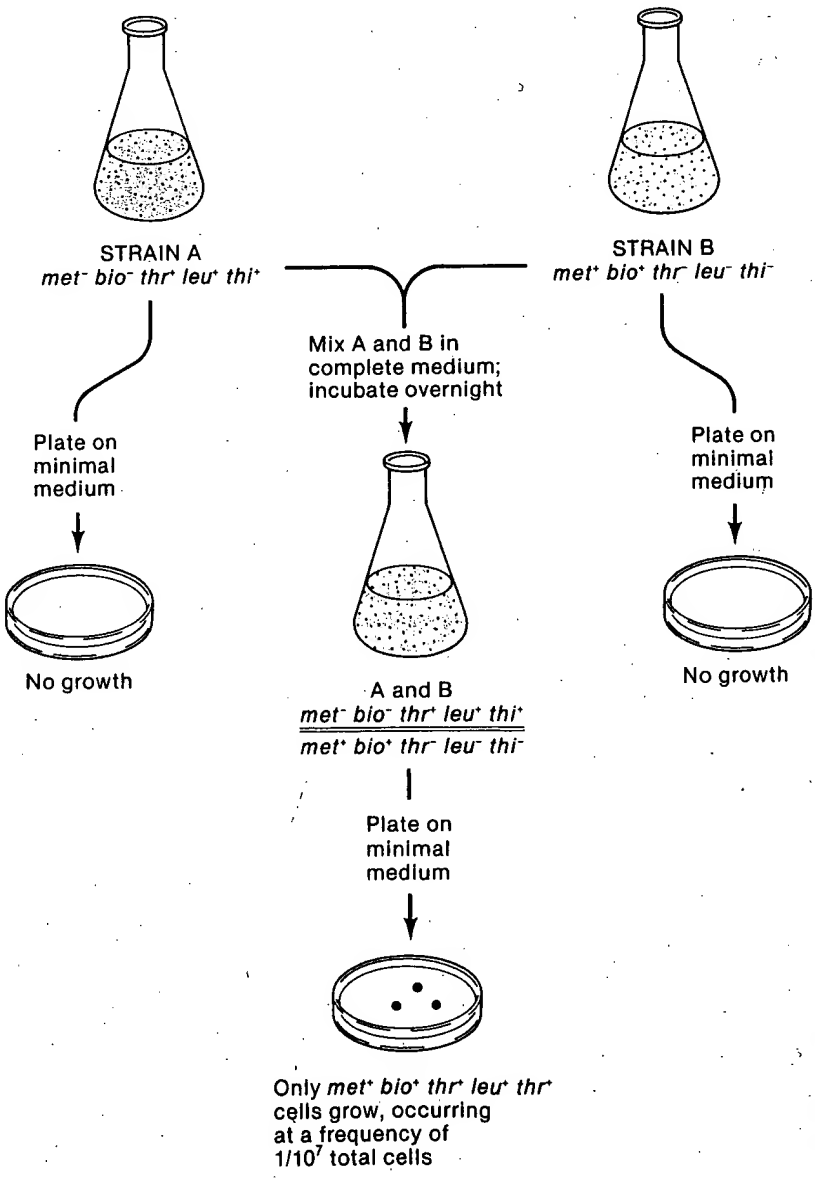

FIGURE 14.6
Recombination between two auxotrophic strains resulting in prototrophs. Neither auxotroph will grow on minimal medium, but prototrophs will.

BACTERIAL CONJUGATION

Lederberg and Tatum's initial experiments were performed with two multiple auxotrophic strains of *E. coli* K12. Strain A required methionine and biotin in order to grow, while strain B required threonine, leucine, and thiamine (Figure 14.6). Neither strain would grow on minimal medium. The two strains were first grown separately in supplemented media, and then cells from both were mixed and grown together for several more generations. They were then plated on minimal medium. Any bacterial cells that grew on minimal medium would be prototrophs. It was highly improbable that any of the cells that contained two or three mutant genes would undergo spontaneous mutation simultaneously at two or three locations. Therefore, any prototrophs recovered must have arisen as a result of some form of genetic exchange and recombination.

In this experiment, prototrophs were recovered at a rate of $1/10^7$ (10^{-7}) cells plated. The controls for this experiment involved separate plating of cells from the strains A and B on minimal medium. No prototrophs were recovered. Lederberg and Tatum therefore concluded that genetic exchange had occurred!

In the following decade, many experiments designed to elucidate the mechanism of bacterial recombination were performed. Because the description of these experiments is lengthy and the results and conclusions complex, they will be discussed in a stepwise fashion.

F^+ and F^- Bacteria: Observations and Conclusions

1 Lederberg and Tatum showed that filtrates of lysed cultures of either strain A or B would not lead to the production of prototrophs when added to intact cells of the other strain. Therefore, it appeared that the cells must interact directly for genetic recombination to occur. This idea received strong support from an experiment by Bernard Davis using a U-tube (Figure 14.7). At the base of the tube was a sintered glass filter with a pore size that allowed the passage of the liquid medium, but was too small to allow the passage of bacteria. Strain A was placed on one side and strain B on the other side of the filter. The medium was pulled back and forth by suction during bacterial incubation. Samples from both sides of the tube were then plated on minimal medium, but no prototrophs were found. Thus, it was concluded that **physical contact is essential to genetic recombination.** This physical interaction is the initial stage of the process of conjugation and is mediated through structures called **pili.** Bacteria often have many pili, which are microscopic extensions of the cell. After contact has been initiated, a **conjugation tube** is formed between mating pairs (Figure 14.8), and transfer of DNA begins. While *pilus* and *conjugation tube* are not synonymous terms, some workers believe that a single sex pilus may give rise to a conjugation tube. Others disagree, however.

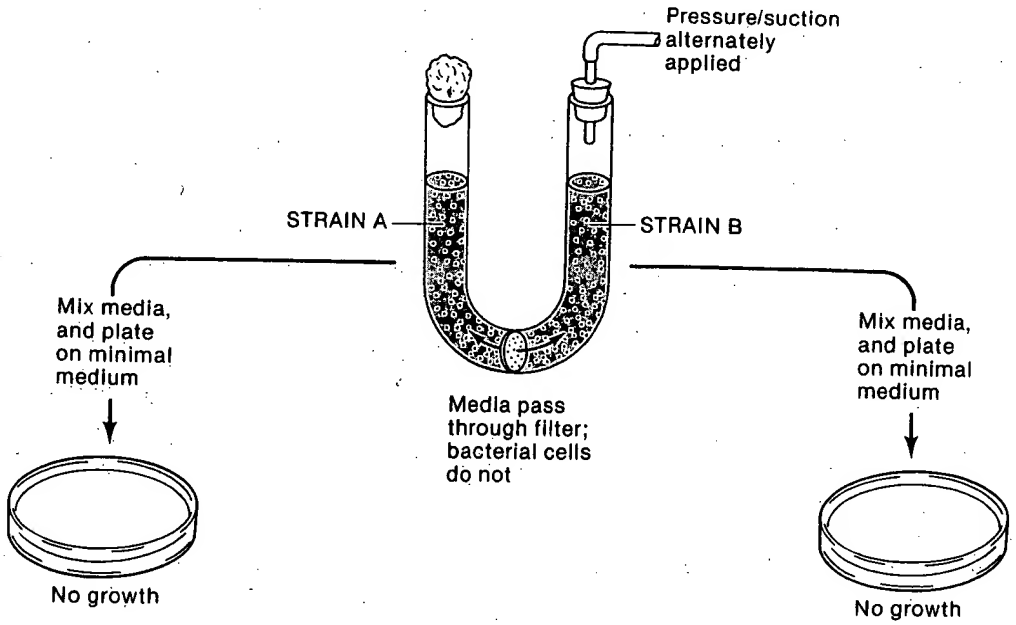

FIGURE 14.7
When strain A and B auxotrophs are grown in a common medium but are separated by a filter, no recombination occurs and no prototrophs are produced. This arrangement is called a Davis U-tube.

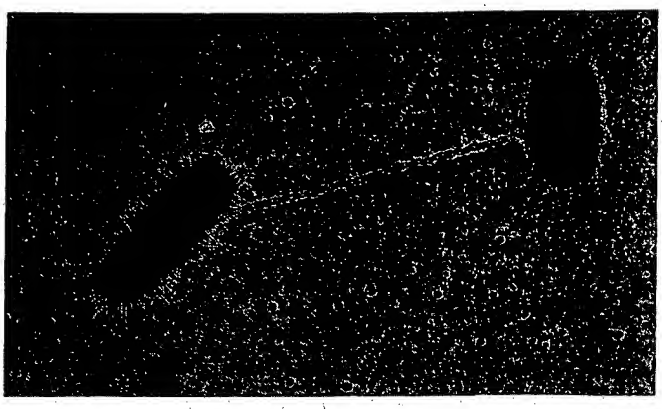

FIGURE 14.8
Conjugation in *E. coli.* The bacterial cell covered with numerous appendages is a genetic donor (F^+) connected to a recipient cell (F^-), which lacks the appendages. The appendages are called pili, and the connection between the cells is the F pilus, sometimes called the conjugation tube. Its formation by the F^+ cell is under the control of genetic information contained in the cytoplasmic F plasmid. The F pilus is labeled along its length by specific virus particles that infect donor cells through this pilus. (Reproduced with the permission of Charles C. Brinton, Jr., and Judith Carnahan.)

2 The initial interpretation of sexuality in bacteria was based on what was already known about single-celled algae and fungi: cell contact, fusion of nuclei forming a diploid zygote, and a meiotic division reestablishing haploidy. In 1952, William Hayes performed experiments with auxotrophs similar to those used by Lederberg and Tatum. His findings established that **transfer of the genetic material is unidirectional.** Hayes found that the number of prototrophs was not diminished greatly if strain A cells were inactivated, or sterilized, by exposing them to high concentrations of streptomycin prior to the cross. Streptomycin is a potent antibiotic that inhibits protein synthesis and prevents subsequent cell divisions. However, no prototrophs were recovered when strain B was similarly treated with streptomycin.

The conclusion drawn was that conjugation involves a **donor** and a **recipient** cell. In Hayes' experiment, cells of strain A were serving as donors, and cells of strain B were the recipients. The reasoning used in drawing this conclusion was that streptomycin, while inhibiting bacterial reproduction of the donor cells, will not prevent them from transferring the genetic material to the recipient. On the other hand, by inhibiting the growth and division of cells serving as recipients, streptomycin effectively terminates the process leading to the recovery of prototrophs. Therefore, these observations support the concept of the occurrence of a nonreciprocal transfer of the bacterial chromosome where cells of strain A serve as "male" donors and those of strain B serve as "female" recipients.

3 In subsequent independent experiments performed by Hayes, the Lederbergs, and Luca Cavalli-Sforza, it was shown that certain conditions could eliminate donor ability in otherwise fertile cells. However, if these cells were then grown with fertile donor cells, fertility was reestablished. The conclusion drawn was that a **fertility factor,** or **F factor,** controls donor ability. It can be lost, converting the donor cell to a recipient, and can also be regained during conjugation. Cells containing the F factor are designated $\mathbf{F^+}$, and those lacking it are designated $\mathbf{F^-}$. On this basis, the initial crosses of Lederberg and Tatum can be clarified:

Strain A		Strain B
F^+	×	F^-
Donor		Recipient

4 **The F factor has been subsequently shown to consist of a circular, double-stranded DNA molecule.** It exists independently from the bacterial chromosome and contains 6×10^4 nucleotide base pairs. This amount of DNA, equivalent to about 2 percent of that making up the bacterial chromosome, is sufficient to code for about 11 genes. Eight of these are involved in the formation of the **sex pilus** that initiates contact between an F^+ and an F^- cell.

5 Following conjugation, **virtually every recipient cell becomes $\mathbf{F^+}$,** suggesting that the F factor is passed through the conjugation tube during each mating, as diagramed in Figure 14.9. In only about $1/10^7$ cells, however, is any part of the donor's chromosome passed to the recipient cell. Apparently, when this occurs, the F factor carries a small piece of the donor chromosome with it during transfer. The part of the chromosome transferred appears to be a random event. All genes thus have a very low, random probability of entering the recipient cell. Following conjugation, the recipient bacterium is partially diploid for the genes transmitted, since two copies are now present. This creates a temporary condition that may lead to genetic recombination. For recombination to occur, the donor genes must be exchanged with the ho-

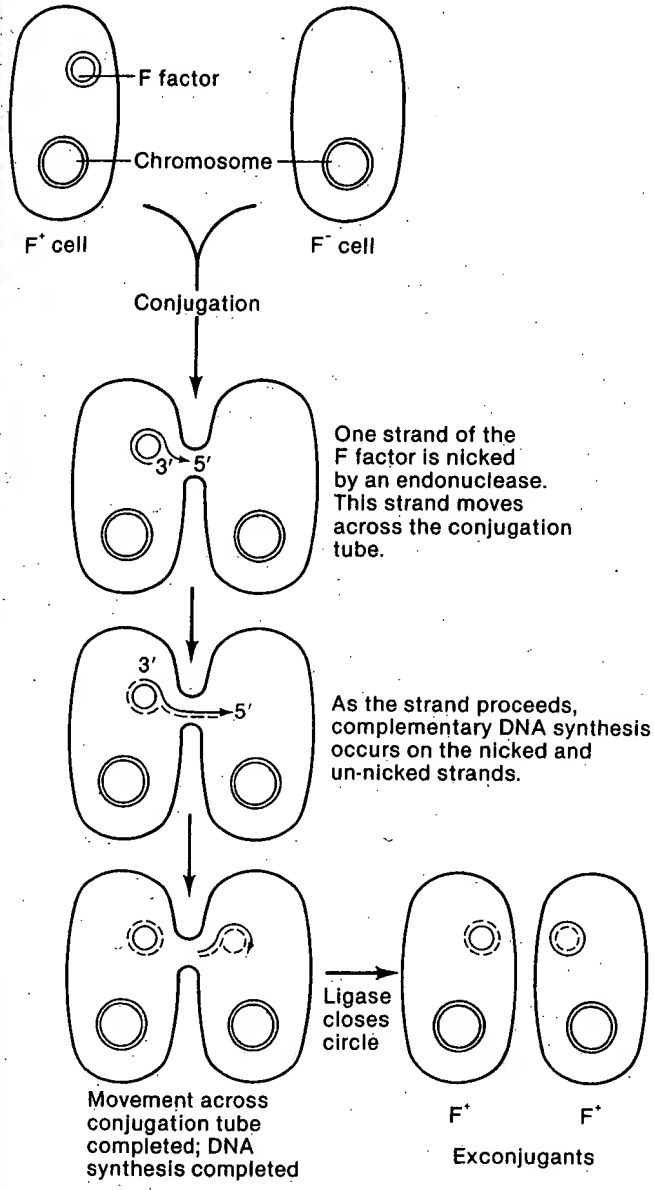

FIGURE 14.9
An $F^+ \times F^-$ mating demonstrating how the recipient F^- cell is converted to F^+. The F factor is transferred across the conjugation tube during its replication.

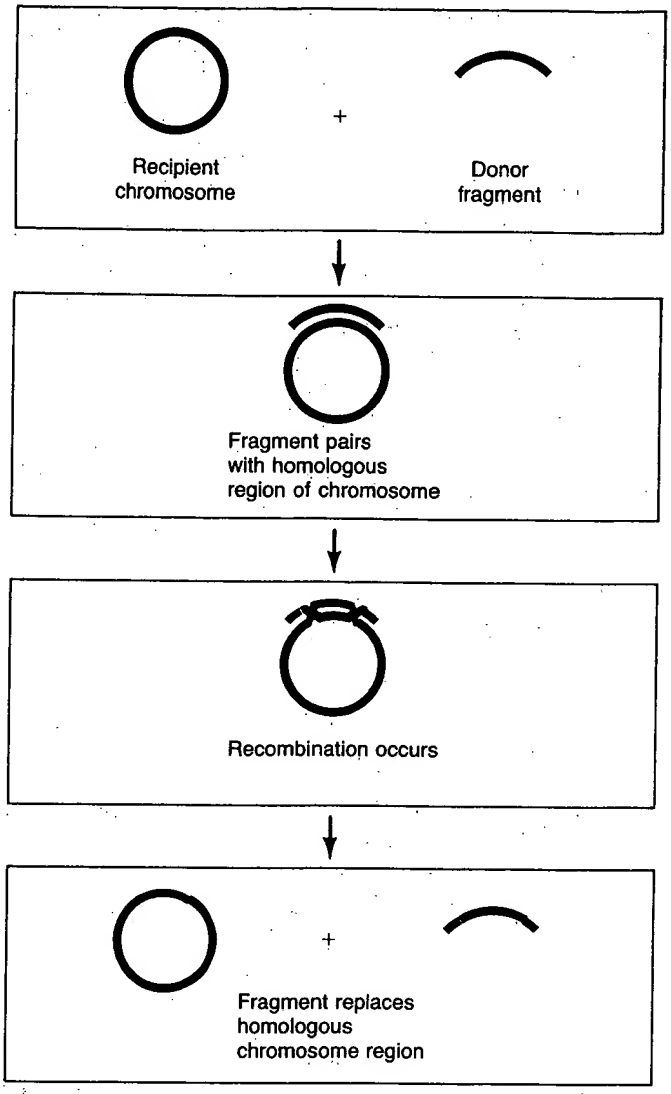

FIGURE 14.10
One possible mechanism by which a piece of a donor chromosome can recombine with the recipient chromosome in an $F^+ \times F^-$ mating.

mologous region of the recipient chromosome. This exchange is most simply visualized as an event involving two crossover sites (Figure 14.10). As a result, the donor genes are integrated into the recipient chromosome, and a fragment of this chromosome is produced. In most cases, the fragment is degraded in the cell.

F^+ and F^-: Summary

In *E. coli*, all cells are either F^+ or F^-, depending on the presence or absence of a small, circular DNA fragment called the F factor. When this factor is present, the cell is able to form a conjugation pilus and serve as a donor of genetic information. During conjugation, a copy of the F factor is always transferred to the F^- recipient, converting it to the F^+ state. Only infre-

quently is a random portion of the donor chromosome also transferred. When it is, the recipient cell may integrate it into its own chromosome through a recombinational event. This series of events serves as the basis for the conversion of auxotrophic strains to prototrophic strains, as originally observed by Lederberg and Tatum.

Hfr Bacteria and Chromosome Mapping: Observations and Conclusions

Subsequent discoveries revealed that the circumstances surrounding genetic exchange during conjugation are even more complex than those described above. Again, we will approach this topic by listing a series of observations and conclusions, followed by a summary.

1 In 1950, Cavalli-Sforza treated an F^+ strain of *E. coli* K12 with nitrogen mustard. From these treated cells he recovered a culture of donor bacteria that underwent recombination at a rate of $1/10^4$ (10^{-4}), 1000 times more frequently than the original F^+ strains. In 1953, Hayes isolated a similar strain that yielded recombinants at a similar frequency. Both strains were designated **Hfr,** or **high-frequency recombination.** Since Hfr cells behave as donors, they are a special class of F^+ cells.

2 Another important difference was noted between Hfr strains and the original F^+ strains. The recipient cells, while sometimes displaying genetic recombination, rarely became Hfr; they remained F^-. In comparison, then,

$$F^+ \times F^- \rightarrow F^+$$
$$Hfr \times F^- \rightarrow F^-$$

3 Perhaps the most significant characteristic of Hfr strains is the nature of recombination. While an F^+ cell donates a random segment of its chromosome, genetic recombination involving an Hfr cell is strain-specific. In any given strain, certain genes are more frequently recombined than others, and some not at all. This **nonrandom pattern** was shown to vary from Hfr strain to Hfr strain.

4 While these results were puzzling, Hayes interpreted them to mean that some physiological alteration of the F factor had occurred, resulting in the production of Hfr strains of *E. coli.* In the mid-1950s, experimentation by Ellie Wollman and Francois Jacob clarified the basis of Hfr and showed how these strains allowed genetic mapping of the *E. coli* chromosome. In their experiments, Hfr and F^- strains with suitable marker genes were mixed and recombination of specific genes assayed with time. To accomplish this, cultures of an Hfr and an F^- strain were first incubated together. At various

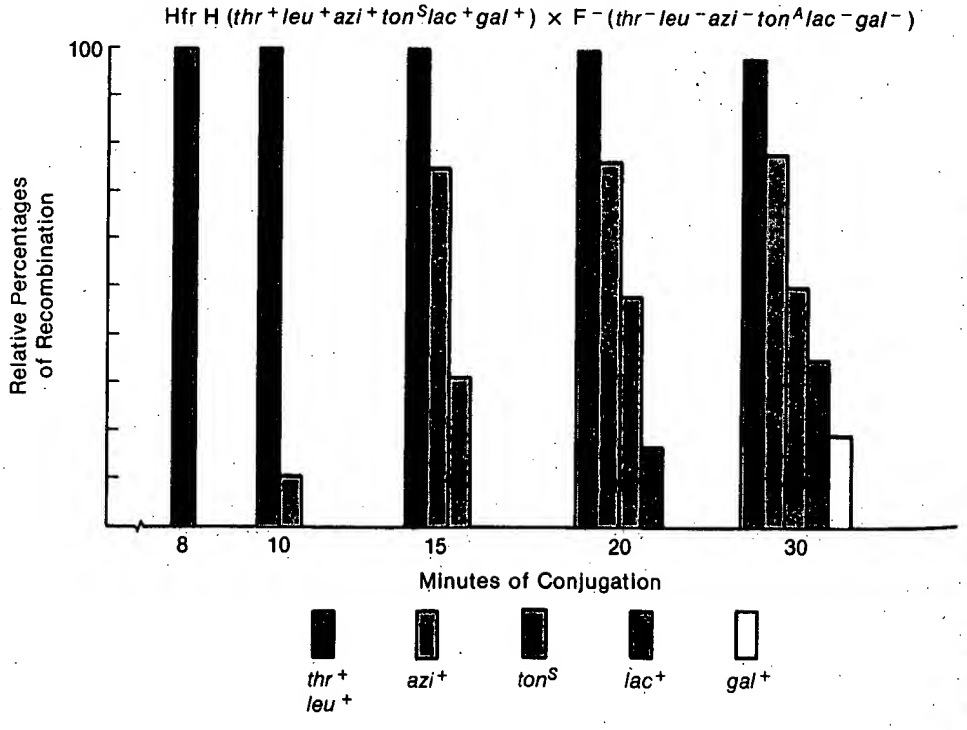

FIGURE 14.11
The progressive transfer during conjugation of various genes from a specific Hfr strain of *E. coli* to an F^- strain. Certain genes (*thr*$^+$ and *leu*$^+$) are quickly transferred and recombine with high frequency. Others (*lac*$^+$ and *gal*$^+$) take longer to be transferred and recombine with a lower frequency.

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